cAMP Influence on Brain and Germinal Cells RNA Syntheses in *Lithobius forficatus* (L.): an Autoradiographic Study

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ABSTRACT

Mature *L. forficatus* were injected with 2 nmol of dibutyryl cAMP (sodium salt) [dBcAMP]. Animals of day 1, 2, 3 and 7 after injection were investigated. Autoradiographs were analysed either by cytophotometry (Leitz MPV) or by image analysis (Biocom 2000 device). *Germinal cells*: in oocytes, the supply of dBcAMP led to a significative increase in [^{3}H]-uridine uptake during the first 3 days of the experimental series. Nevertheless, on day 7, control values were obtained. In spermatocytes, two cases must be reported: if the testes were not in a phase of active spermatogonial divisions ("phase de reconstitution"), an increase in RNA syntheses was observed. At the opposite of that measured for oocytes, the maximum of label was observed on day 7. For testes with active mitoses, the uptake remained at a low level, either in controls or in dBcAMP injected animals. *Brain*: analysis performed on various areas (middle and lateral parts of the so-called *pars intercerebralis*, frontal lobes and cerebral glands) showed that dBcAMP has a stimulating effect on the uptake of the tritiated precursor. The increase of RNA syntheses observed in germinal cells and in neurons shows that cAMP is a good candidate to be the second messenger of the stimulating neuropeptide(s) issued from *pars intercerebralis* neurosecretory cells. Our results have, in addition, shown that a refractory period occurs during the period of active mitoses in the testis.

RÉSUMÉ

Influence de cAMP sur la synthèse de l'ARN du cerveau et des cellules germinales chez *Lithobius forficatus* (L.): étude autoradiographique.

Après injection de 2 nmol de dibutyryl cAMP (sel de sodium) [dBcAMP], des adultes matures de *Lithobius forficatus* ont été étudiés aux jours 1, 2, 3 et 7. Les autoradiographies analysées, soit par cytophotométrie, soit par un analyseur d'images Biocom 2000, montrent que les ovocytes incorporent significativement plus d'uridine tritiée que les témoins durant les 3 jours qui suivent l'injection de dBcAMP. Des valeurs témoins sont cependant récupérées au jour 7. En ce qui concerne les spermatocytes, deux cas se présentent : si le testicule n'est pas en phase de reconstitution (période de divisions goniales), une augmention des synthèses d'ARN est observée. Aucune augmentation de synthèse n'est observée si de nombreuses divisions goniales sont en cours dans le testicule. Les analyses effectuées sur différentes zones du cerveau (*pars intercerebralis*, lobes frontaux) ou sur la glande cérébrale, montrent un effet stimulateur sur l'incorporation

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du précurseur tritié. L'augmentation des synthèses d'ARN observée aussi bien dans les cellules germinales que dans le système nerveux montre que l'AMP cyclique est sans doute le second messager du (des) neuropeptide(s) issu(s) des cellules neurosécrétrices de la *pars intercerebralis*. Nos résultats montrent en outre l'existence d'une période réfractaire à l'action de l'AMP cyclique.

INTRODUCTION

In *Lithobius*, the endocrine control of gametogenesis is the result of the balance between stimulating factors - ecdysteroids and a hormone issued from *pars intercerebralis* neurosecretory cells (pi NSC) - and a moderating one released from the cerebral glands (neurohemal cephalic organs) (reviews: JOLY & DESCAMPS, 1988; DESCAMPS, 1992a). In addition, in females, it has been shown that too high levels of ecdysteroids triggers the release of the moderating factor (DESCAMPS, 1992b). It has been previously demonstrated that cAMP was present in testes and that the level of this messenger was increased, except during meiosis, after electrical stimulation of the pi NSC (DESCAMPS *et al*, 1986). So, it was of interest to proof the effects of cAMP on germinal cells and on brain neurons metabolism, in order to compare the results to those obtained after electrical stimulation or ecdysteroid injection.

MATERIAL AND METHODS

The experiments were conducted on mature *Lithobius forficatus* (L.) collected in northern France, during autumn, in order to have animals showing a low rate of RNA syntheses, at least in male germinal cells.

Animals were injected with 2 nmol of dibutyryl cAMP (sodium salt) [= dBcAMP; purchased from Sigma] in solution in a saline adapted to chilopods. The autoradiographic study was conducted after the injection of 185 KBq (= 5 µCi) of [^{3}H]-uridine (CEA, France; specific activity 166,5 TBq [= 45 Ci]/mMol) 48 hrs before fixation. The animals were fixed 1, 2, 3, and 7 days after the injection of the dBcAMP. Tissue sections, treated according to FICQ (1961), were covered with Kodak NTB3 emulsion. Kodak D19 was used as developer. The cytophotometric study of the labelling of germinal cells was conducted either with a Leitz MPV cytophotometer, using a 500 µm^2 diaphragm. Only growing spermatocytes (40 to 70 µm in diameter) or vitellogenetic oocytes (stage 2B and beginning of stage 3 according to HERBAUT, 1972) were investigated. Brain endocrine areas were studied with an image analysis device (Biocom 2000). In the graphs concerning the lattest results no error bars are shown, measurements being the results of area labelling expressed as a mean percentage of label of the area (neuropil label is defined as an internal control and fixed to 1) and not as the result of cell types measured individually.

RESULTS

Germinals cells

In oocytes, a significant increase of uptake of [^{3}H]-uridine was recorded in animals of day 1, 2, 3. In day 7 animals the label was comparable to that of the controls (Fig. 1).

In males, two cases were recorded. In animals with a testis showing growing spermatocytes, an increase of uptake was present in all the experimental series (Fig. 2). In the animals that undergo the renewal of their stock of spermatocytes ("période de reconstitution", JOLY & DESCAMPS, 1969), at the opposite of that observed precedently, the level of label was low in controls (*circa* 30 units), and in addition, no effect of dBcAMP was found (label staying between 20 and 25 units).

Brain

In all females, an increase uptake of the tritiated precursor was found in the endocrine areas [median (Fig. 3) and lateral (Fig. 4) parts of the pi, frontal lobes (Fig. 5)]. Nevertheless, the maxima recorded here were different according to the type of NSC studied.

The results were quite different for the cerebral glands. An increased uptake took place during the first three days, a control value was measured from day 7 animals (Fig. 6).

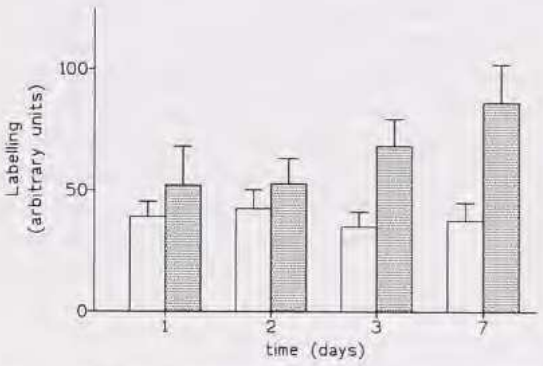


FIG. 1. — Cytophotometric measurements of labelling over oocyte nuclei after 1, 2, 3, 7 days. Means ± SD. Controls: open bars.

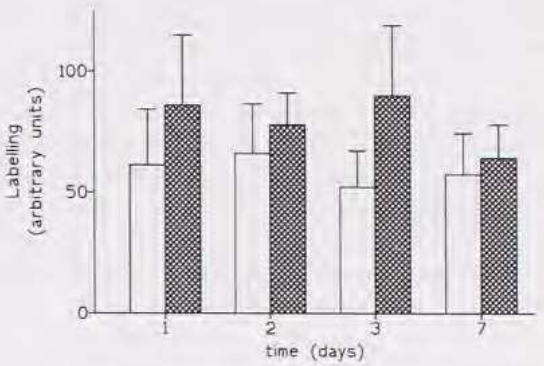
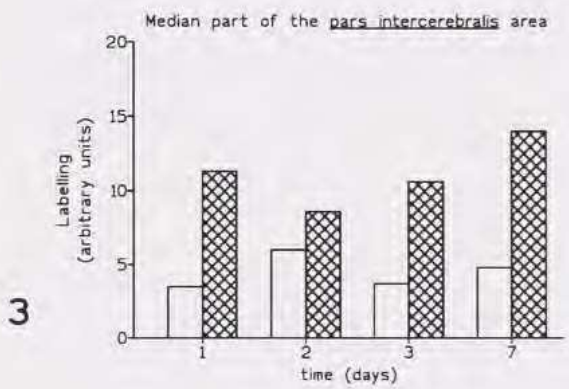
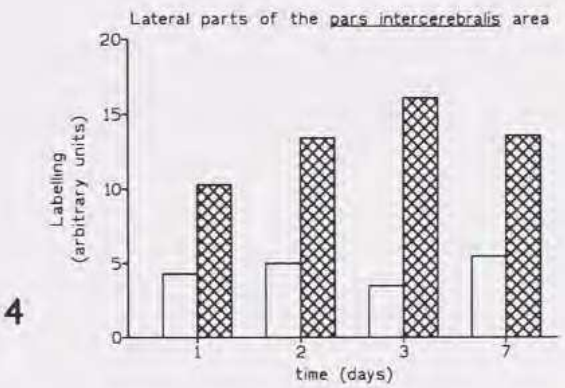
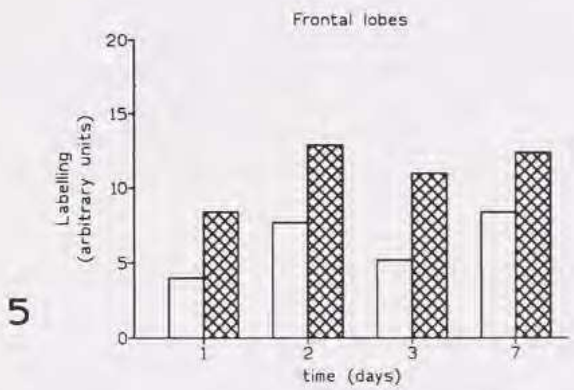
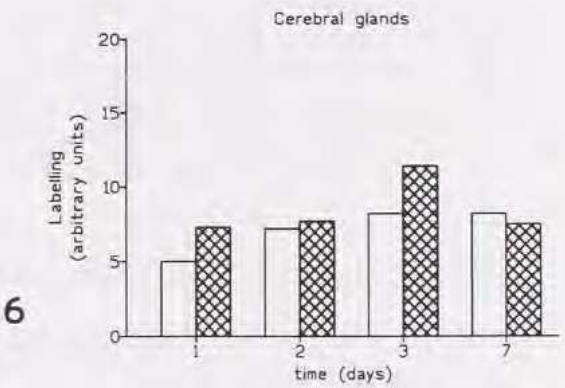


Fig. 2. — Cytophotometric measurements of labelling over spermatocyte nuclei after 1, 2, 3, 7 days. Means ± SD. Controls: open bars.



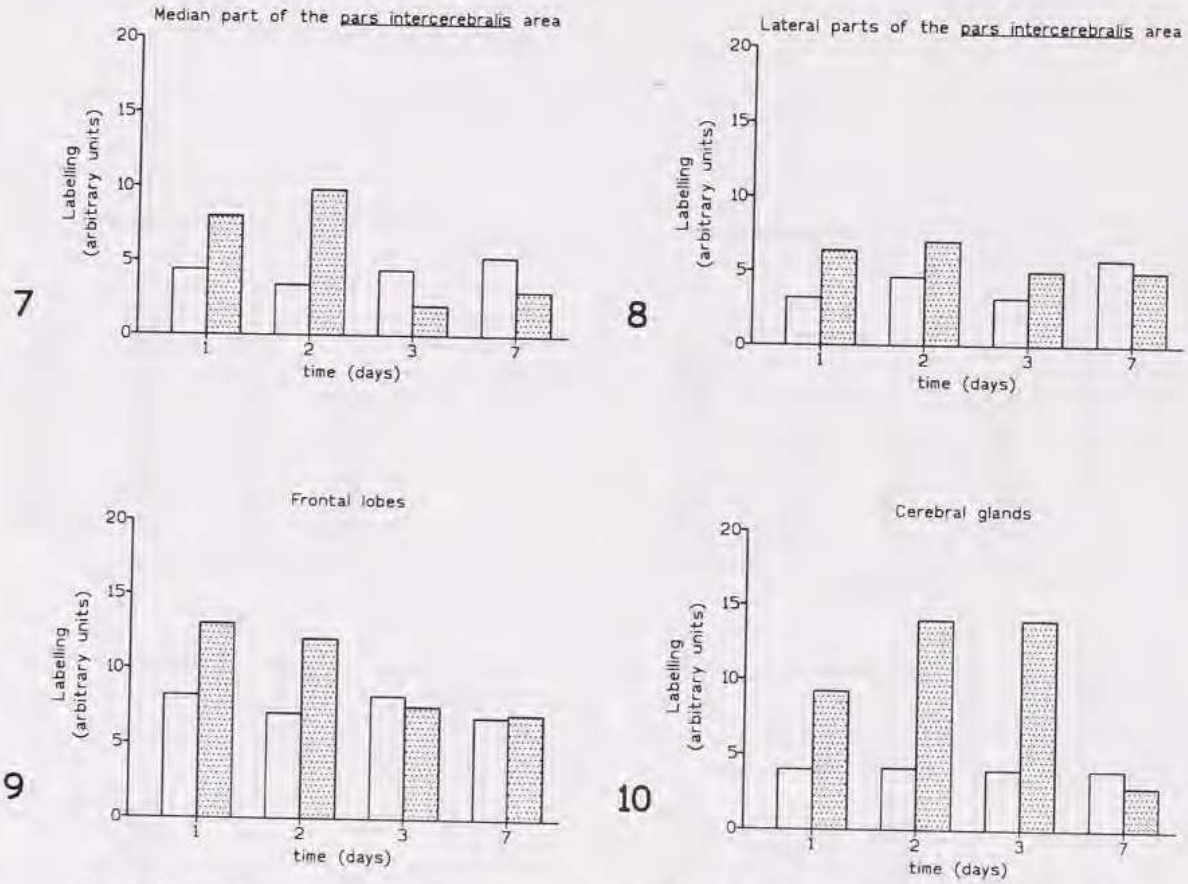






FIGS. 3-6. — Mean label of brain endocrine areas of pi and of cerebral glands in females. Controls: open bars.

In males, we can only report the results concerning animals not implied with the renewal of the spermatocyte stock. As a consequence, the label was low over the brain and cerebral gland, but not strong enough to enable measurements by the image analyser. In animal not concerned with this refractory period, an increase in the uptake of [^{3}H]-uridine was observed, but for shorter times than in females. For the median part of the pi, this increase lasted two days. Then, the values recorded were significantly under the control values (Fig. 7). Lateral parts of the pi showed, compared to the controls, increases of uptake, but only during the first three days; nevertheless, it must be noticed that these increases were of less great extend than those recorded for the median part (Fig. 8). In frontal lobes labelling was increased only in the first two days and then control values were recorded (Fig. 9). The most dramatic increases were recorded for the cerebral glands, particularly for animals of day 2 and day 3. In day 7 animals, values recorded were slightly under the control values (Fig. 10).



FIGS. 7-10. — Mean label of brain endocrine areas and of cerebral glands in males. Controls: open bars.

CONCLUSIONS AND DISCUSSION

As a general conclusion, we can say that cAMP stimulates the uptake of [^{3}H]-uridine and can be considered as a good candidate to be the 2nd messenger of various kind of cells as those of NSC or germinal cells.

Stimulation of uptake in oocytes is only transient: this result can be compared to findings after injection of (at least) 0.4 µg of 20-hydroxyecdysone and was explained by the release of a moderating factor, in order to limitate the level of the metabolism and to enable a normal and regular vitellogenesis (DESCAMPS, 1992b). So, also in this case, the release of a moderating factor is more likely involved in the regulatory process, explaining as a consequence the rather short time of increased metabolism in oocytes.

At the opposite, stimulation of spermatocyte metabolism was observed during the whole time of experiment, comparable to previous results (DESCAMPS, 1981, 1991). It appears that, either there is no release of a moderating factor in males, the balance between the stimulating and the moderating hormones being not regulated in the same manner in males and in females, or, as another explanation, the spermatocytes are less sensitive to variations of hormonal levels. They might be protected by the testis blood barrier, the lattest being regulated partly by 20-hydroxyecdysone (BENIOURI, 1984).

Concerning the brain, the increase of tritiated uridine uptake in pi NSC requires more time in females (at least 7 days) than in males (2 or 3 days). This fact can be related to the reproductive physiology of animals: females are in their vitellogenetic phase whereas males are entering in a period of minimal rate of metabolism (the so called winter rest period). Differences between female and male brains were previously evidenced in transplantation experiments on the influence of the *pars intercerebralis* on the gametogenetic cycle (DESCAMPS, 1974), and, at this time, it was suggested that these differences took their origin in the course of gametogenetic cycles. The present results are in full agreement with these statements.

In the frontal lobes, increased values were recorded during the whole time of experimental series for both sexes, whereas in cerebral glands an increase was measured for only the first three days. It is difficult to explain or to relate these findings to physiological events: release of moderating factor by the cerebral gland do not imply increased syntheses, numerous secretory granules being stored in the cells and in the axonal endings of the glands. In short, for the brain, differences are only recorded according to the sex, and this was previously reported in transplantation experiments (DESCAMPS, 1974).

cAMP is involved in various kind of processes. For example, in Crustacea it has been found that the processes of protein synthesis are involved in previtellogenic oocytes (EASTMAN-REKS & FINGERMAN, 1984) and those which are necessary for ecdysteroid production are inhibited by MIH (molt inhibiting hormone) through cAMP (among other authors: MATTSON & SPAZIANI, 1985; SEDLMEIER & FENRICH, 1993). At the opposite, in Insecta, the process of ecdysteroid synthesis is stimulated by PTTH (prothoracotropic hormone) through cAMP (among others: SMITH *et al.*, 1984, 1993).

We have found that, in the brain, stimulating or moderating factors of NSC secreting show both an increased uptake of [^{3}H]-uridine after dBcAMP supply. It is the question if the increase of this uptake that is triggered in NSC frontal lobes is directly induced by the cyclic nucleotide or if the activation of NSC frontal lobes is a consequence of the activation of pi NSC in order to counteract their action in a regulatory process of metabolism? The answer to such a question cannot be given before the localization of adenylate cyclase will be investigated.

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